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In-Vitro Antibacterial Activity of Ruta Chalepensis (Tenadam) and Justicia Shimperiana(Senel) Plants against Some Bacterial Human Pathogens

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Abstract
The leave extracts of *Ruta chalepensis* and *Justicia schimperiana* were the most powerful medicinal value. The plant extraction followed by ethanol, methanol acetone, diethyl ether and hexane by using disc diffusion and broth dilution methods (MIC) against six human pathogenic bacterial strains (*Shigella dysentery, Escherichia coli, Streptococcus aeruginosa, Pseudomonas aeruginosa, Staphylococcus aureus, Salmonella typhi* and *Klebsiella pneumonia*). The methanol extract of *J. schimperiana* showed strong inhibition activity against *S. dysentery* and *E. coli* with a zone size of 14.5±0.5mm and 16±0.2mm and MIC values of 3.12mg/ml against *E. coli* and *S. dysentry*. Highly prominent activity was produced by the ethanol extract of *R. chalepensis* with the highest zone of 15±0.5mm diameter observed in *S. typhi*, followed by *S. aureus*13±0.11mm with the MIC value of 1.56mg/ml against *S. typhi.* Four antibiotics were used as standard for the testing of antibacterial activity against six different human pathogens. Among the antibiotics Ciprofloxin showed maximum zone of inhibition ranging from 20-35mm followed by Kanamycin, Tetracycline and Chlormphenicol.
Keywords: *Antibacterial activity, Broth dilution, Disc diffusion, Human pathogens*
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INTRODUCTION

The discovery and development of antibiotics are among the most powerful and successful achievements of modern science and technology for the control of infectious diseases. The number of multi-drug resistant microbial strains presented (Blair et al., 2015). There is an urgent need to control antimicrobial resistance by improved antibiotic usage and reduction of hospital cross-infection. However, the development of new antibiotics should be continued as they are of primary importance to maintain the effectiveness of antimicrobial treatment (Marchese and Shito, 2001).

The potential for developing antimicrobials from higher plants appears rewarding as it is lead to the development of a phytomedicine to act against microbes; as a result, plants are one of the bedrocks for modern medicine to attain new principles (Evans *et al.,* 2002). Plant based antimicrobials have enormous therapeutic potential as they can serve the purpose without any attributable side effects that are often associated with synthetic antimicrobials. Further continued exploration of plant derived antimicrobials is current needed (Hussain and Gorsi, 2004). Medicinal plants constitute an effective source of both traditional and modern medicines. Herbal medicine has been shown to have genuine utility and about 80% of rural population depends on it as primary health care (Akinyemi *et al.,* 2005).

In recent years, pharmaceutical companies have spent a lot of time and money in developing natural products extracted from plants, to produce more cost effective remedies that are affordable to the population (Doughari, 2006). The screening of plant extracts and plant products for antimicrobial activity has shown that plants represent a potential source of new anti-infective agents (Salvat *et al.,* 2001; Costa *et al.,* 2008). Numerous experiments have been carried out to screen natural products for antimicrobial property (Ateb and Erdourul, 2003; Nair and Chanda, 2006; Nair *et al.,* 2007; Ndhlala *et al.,* 2009. Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant (Nascimento *et al.,*2000).

Ruta chalepensis is strongly scented evergreen sub shrubs 20–60 cm tall. The leaves are bi pinnate or trip innate. The flowers are yellow, with 4–5 petals, about 1 cm diameter, and borne in cymes. The fruits of the plant are 4-5 lobed capsules, containing numerous seeds. Traditionally, used as remedy for many inflammatory diseases and as an antispasmodic, as a treatment for menstrual problems, as an abortifacient and as a sedative and for the treatment of rheumatism and mental disorders. In Ethiopia, the leaves are used for myalgia, cold, whooping cough, abdominal pain, anti-emetic, inflammatory diseases, dropsy, neuralgia, rheumatism and menstrual and other bleeding disorders (Figure 1).

Figure 2A:- *Ruta chalepensis Sample collection (My Original photo, 2017).*

Justicia schimperiana is belonging to the family acanthaceae which is fast growing on the altitude of 8,000m and commonly erect shrub up to 4 m high. The stem is woody and with internodes; leaves decussate, estipulate, simple, ovate-oblong in outline; inflorescence thyroid, with densely flowered spikes; corolla bilabiate white to creamy white; fruit capsule**.** J. schimperiana are traditionally used for the treatment of diabetes mellitus, stomach-ache and burning, constipation and tooth ache and use leafs by mixed with local beer as a remedy against bronchial asthma. Northern Ethiopia the plant alone or in combination with other plants is used for various diseases such as epilepsy, mental illness, eye diseases, jaundice, malaria, leprosy, syphilis, gonorrhea, rabies, measles, relapsing fever, vitiligo, gout and acute febrile illness. In Southwest Ethiopia, it is used for malaria, scabies, where the fresh leaves are crushed and macerated in water and then the affected area is washed with the macerate **(**Teferi, 2003).

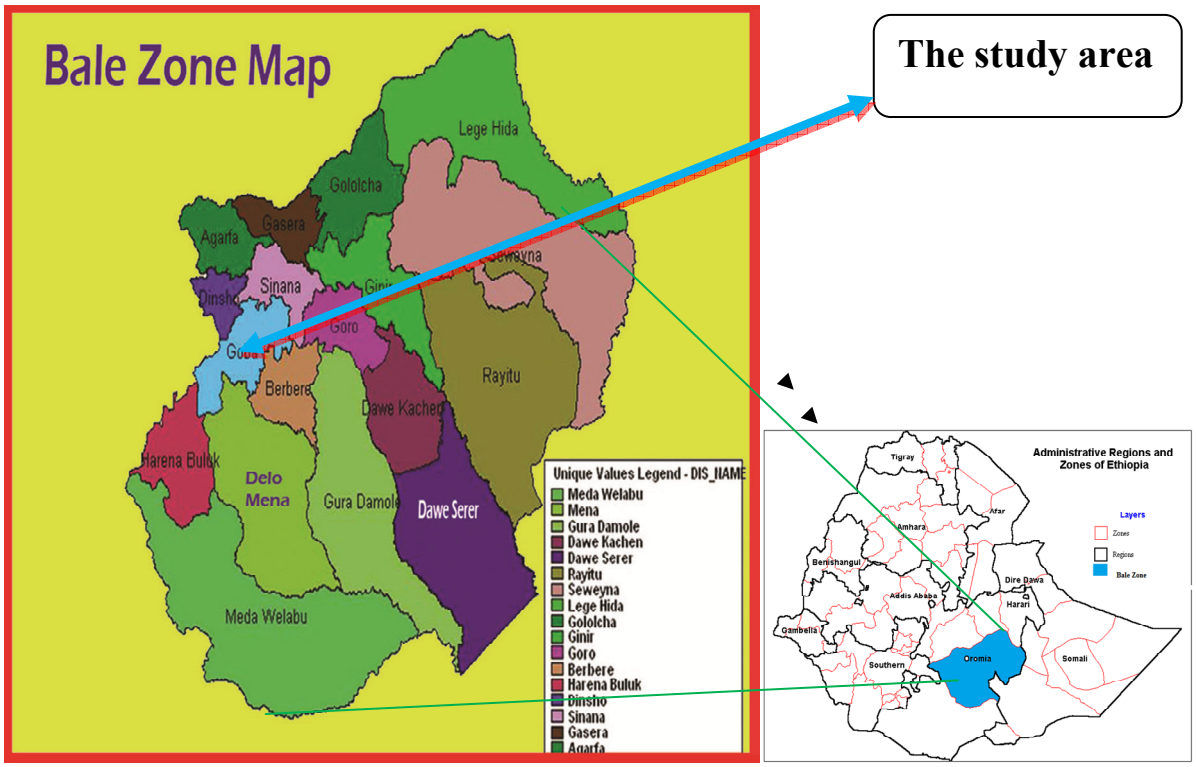
Figure 1B:- *Justicia schimperiana sample collection (My Original photo, 2017).*

In Ethiopia, plant remedies are still the most important and sometimes the only sources of therapeutics for nearly 80% of human and more than 90% in livestock population. Estimated floras of 6500 to 7000 species of higher plants are found in Ethiopia. Out of these 12% are endemic to the country (Tadeg *et al.,* 2005: Giday *et al.,* 2009). Despite their vital role in catering for the health of human and livestock population, large part of the knowledge of ethno medicinal plants is on the verge of irreversible loss and declining to deterioration due the oral passage of herbal heritage from generation to generation rather than in writings (Mesfin *et al.,* 2009). Environmental degradation, agricultural expansions, cultivation of marginal lands and urbanization are also posing a significant threat to the future wellbeing of human and animal populations that have relied on these resources to combat various ailments for generations (Lulekal *et al.,* 2008: Devi *et al.,* 2009).

Materials and Methods

Location of study area

The study was carried out on some medicinal plants collected from Goba districts of Bale zone, Oromia Regional State, South Eastern Ethiopia. Goba district was located at 445 km south east of Addis Ababa. The area was situated at 7º00’ N and 39º58’ E Latitude and longitude respectively. The area has a typical vegetation type of undifferentiated Afromontane forests in Ethiopia and has a mean annual rainfall and temperature of 1218.64 mm and 10.26 ºC, respectively. The economic activities of the local people were primarily based upon mixed farming that involves pastoralism and cultivation of crops such as wheat and barley.



Map of the Study Area

Collection and identification of plant materials

Two medicinal plants, *R. chalepensis* and *J. schimperiana*, were collected from Bale Zone, Goba district Oromia region, Ethiopia. The taxonomic position of the plants were identified and authenticated by plant experts from National Herbarium in Addis Ababa University. Leaves from the study plants were taken in a large quantity and repeatedly washed under tap water to remove any debris and were air dried under shade for fifteen days.

Preparation of plant's crude extracts

The preparation of crude extracts of plants under this study was conducted following the methods used by Hailu Tadeg *et al*. (2005) using different solvents. Five hundred grams of leaves from each plant was taken for extraction procedure and ground in a mortar and pestle separately under aseptic condition. Twenty grams of each powdered plant material were extracted with apparatus containing 250 ml of Each solvent of ethanol, methanol, diethyl ether, hexane and acetone separately by maceration for 48 h with frequent agitation on orbital shaker continuously for two days and the resulting liquid was filtered (Whatman No. 3 filter paper, Whatman Ltd., England). Extraction was repeated five times and the filtrates of all portions were combined in one vessel. The organic solvent was removed by evaporation using Rota vapor (BU¨CHI Rota-vapor R-205, Switzerland) at no more than 40 ºC.

The solvent residue was then placed in an oven at 40 ◦C for about 48 h to remove the solvent. The resulting dried mass was then powdered, packed into a glass vial until use. Finally, the gram yield of dried residue of each plant extracts were calculated. The concentrated extracts were stored at 4°C for the next antimicrobial study. Dried residues were dissolved in 100 % dimethyl sulfoxide (DMSO) to obtain a stock concentration of 100 mg/ml, which was kept at 4 °C until use.

Preparation of test organisms

The test organisms including standard pathogens were Escherichia coli, Salmonella typhi, Shigella dysentry, Pseudomonas aerugenosa, Staphylococcus aureus and Klebsiella pneumonia were obtained from Ethiopian Public Health Institute (EPHI), Addis Ababa, Ethiopia. These microorganisms were suspended in nutrient broth and sub cultured into fresh nutrient agar medium and kept at 4°C until use. The inoculums preparation were standardized by inoculating bacterial strains from the exponential phase and standardized with 0.5 McFarland turbidity prepared by adding a 0.5 mL aliquot of 1.175% w/v $BaCl_2.2H_2O$, added to 99.5 mL of 0.18 mol/L H_2SO_4 (1% v/v).

Antimicrobial Assay

Antibacterial sensitivity testing using disc diffusion method

For antibiotic susceptibility testing, a stock concentration of (100mg/ ml) plant crude extracts was prepared in

DMSO. A circular antibiotic assay disc of 6 mm diameter was prepared from the Whatman filter paper No.3 and sterilized by autoclaving for 15 min at 121° C. The sterile discs were impregnated with 50µl of the reconstituted extract and were dried completely at 37 °C overnight. A sterile cotton swab was then dipped into a homogenous suspension of test organism with adjusted 0.5 McFarland turbidity standards (Habtamu *et al.,* 2018). The test pathogenic microorganisms were gently spread by streaking onto Muller Hinton Agar (MHA) and then allowed to dry for half an hour. Then the discs were aseptically placed over plates of Muller Hinton Agar (MHA) (Haniyeh *et al*., 2010). The plates were incubated in an upright position at 37 °C for 24 hours and the zone of inhibition measured (in mm diameter). Inhibition zones with diameter less than 12 mm were considered as having low antibacterial activity. Diameters between 12 and 16 mm was considered moderately active, and those with >16mm were considered highly active (Indu *et al*., 2006). The test organisms were tested for their sensitivity against the standard antibiotics, Ciprofloxacin (35 µg), Chloramphenicol (30 µg) Tetracycline (30 µg) and Kanamycin (20µg) by the disc diffusion method according to (Habtamu *et al*., 2019).

Minimum Inhibitory Concentration (MIC) assay methods

The minimum inhibitory concentration (MIC) was determined by comparing the various concentrations of plant extracts which have different inhibitory effect and selecting the lowest concentration of extract showing inhibition (Agatemor, 2009). The MIC was determined for crude extracts that showed inhibition zone of $\geq$ 7 mm diameter and for extract that inhibited the growth of all tested bacteria at concentration of 200 mg/ml. The test was performed by using standard tube dilution (serial dilution) method using nutrient broth as diluents. Accordingly, the plant extract was prepared by double serial dilution from 200 mg/ml to obtain 1:2, 1:4, 1:8, 1:16, 1:32, and 1:64 in order to get 100, 50, 25, 12.5, 6.25, 3.125, 1.56 and 0.78 mg/ml concentration of extract respectively using 50% DMSO. 1 ml of each extracts was dissolved in sterile test tubes which contained 9 ml of nutrient broth. Then, 0.1ml of the test organism was inoculated to the each tube. One tube was used as the control (broth + extract). The tubes were incubated at 37 °C for 24 hrs and the presence of growth was evaluated by comparing the optical density (OD) of each well before and after incubation (Habtamu *et al.,* 2018). When the difference of OD value (after incubation-before incubation) of the test (broth + extract + organism) was greater than that of the control (broth + extract) at each concentration, it was considered as presence of turbidity or growth of bacteria. The lowest concentration, at which there was no turbidity, was also regarded as MIC value of the extract.

Ethical consideration

The study was conducted after its approval by the Department of Biology, School of Natural Science, Madda Walabu University. The tests were done in Applied Microbiology Laboratory, Department of Biology using standard good laboratory practices (GLP) to minimize the risk of exposure to human pathogenic bacteria used in the study.

Data Analysis

Data on mean inhibition zone produced by each plant crude extract and MIC on various bacteria were entered in to Microsoft excels spreadsheet and then exported to SPSS (version 16). The values were given as mean± SD.

RESULTS

Antibacterial activity of the plant extracts

The crude leave extracts of Ruta chalepensis and Justicia Schimperiana were tested for antibacterial activity on six human bacterial pathogens. The solvents that were used in this study produced an overall yield of plant crude extracts that were ranging from 1.1 to 1.8 gm. from different plants (Table.1). The results of study revealed that the tested two medicinal plants Ruta chalepensis and Justicia Schimperiana extracts possess a potential antibacterial activity.

Table 1. The yield of plant crude leave extracts by using different solvents

Plant species	Extraction type	Yield in grams (Mean)
Ruta chalepensis	Methanol	1.4
	Ethanol	1.8
	Diethyl ether	1.2
	Acetone	1.3
	Hexane	1.1
Justicia shimperiana	Methanol	1.8
	Ethanol	1.7
	Diethyl ether	1.3
	Acetone	1.35
	Hexane	1.18

The antibacterial activity of *Ruta chalepensis* crude extracts

The methanolic extract of *Ruta chalepensis* produced a maximum zone of inhibition of 11.1±0.55mm in diameter against *S. aureus.* The *K. pneumoniae, S. dysentry* and *E. coli* exhibited a relatively moderate mean inhibition of 9.5±0.72 mm, 9±0.4 mm and 9±0.3mm, respectively. The other pathogens showed activity with *P. aeruginosa* (8.8±0.26 mm) and *S. typhi* (7±0.5mm.) respectively. The ethanol extract of *R. chalepensis* showed a conspicuous activity against the tested bacterial pathogens. The highest zone of inhibition was observed in *S. typhi* (15±0.5mm in diameter), followed by *S. aureus* (13±0.11mm), *P. aerugenosa* (13±0.2mm) and *E. coli* (12.5±0.5mm) respectively. The least level zone of inhibition was detected against *K. pneumoniae* (7.9±0.11mm*)*.

The acetone crude leave extract of *R. chalepensis* showed a strong zone of inhibition against *K. pneumoniae* (11.1±0.28 mm*)* and a moderate level zone of inhibition against *S. dysentry, S. typhi* and *S. aureus with zone of inhibition* of 8.2±0.3mm, 7.8±0.26mm, 7.5±0.5mm, respectively. *The least antibacterial activity was detected against Escherichia coli with a zone size of* 5.2±0.43 mm. Diethyl ether crude leave extract exhibited a moderate antibacterial activity against *S. dysentry* (7.1±0.32mm) and least activity against *K. pneumoniae* (5.2±0.2 mm*)* and *P. aerugenosa* (51±0.28mm). Hexane extract only inhibited *S. dysentry* with a zone of inhibition of 6.3±0.26 mm.

Table 2 The Effect of the different extracts of the leaves of *Ruta chalepensis* against the bacterial test organism using disc diffusion method (Zones of inhibition in mm; Mean± SD mm**).**

Test Organisms	Mean Inhibition zone of leaves extract* *Ruta chalepensis* (Mean± SD mm)				
	Methanol	Ethanol	Diethyl Ether	Acetone	Hexane
Escherichia coli	9±0.3	12.5±0.51	-	5.2±0.43	-
Salmonella typhi	7±0.5	15±0.5	-	7.8±0.26	-
Shigella dysentry	9±0.4	11±0.1	7.1±0.32	8.2±0.3	6.3±0.26
Staphylococcus aureus	11.1±0.55	13±0.11	-	7.5±0.5	-
Pseudomonas aeruginosa	8.8±0.26	13.7±0.26	5.2±0.2	6.5±0.3	-
Klebsiella pneumonia	9.5±0.72	7.9±0.11	5.1±0.28	11.1±0.28	-

-= implies no inhibition zone detected*; *= *a crude extract of at concemtration of 100mg/ml was used for assay

The antibacterial activity of *Justicia schimperiana* crude extracts

The mean zones of inhibition produced by the methanol extract of *J. schimperiana* on *S. dysentery* and *E. coli* were considerably a higher (14.5±0.5mm and 10.8±0.2 mm) compared to moderate level zone of inhibition exhibited against *S. typhi* (8.7±0.26mm) and *K. pneumoniae* (7.7±0.25 mm). The ethanolic extract of *J. schimperiana* produced the greatest antibacterial activity against all the tested human bacterial pathogens as compared to crude leave extracts by different solvents. With the highest activity produced against *S. dysentry* with the zone size of 16±0.2mm, followed by *K. pneumoniae* (14.8±0.2mm), *S. typhi* (14.7±0.3mm), *S. aureus (*13.1±0.38 mm) and *E. coli* (12.4±0.2mm). Moderate antibacterial activity was exhibited by *P. aerugenosa* (10.8±0.28 mm). No least activity observed in the case of ethanolic extract of *J. schimperiana*.

Acetone extract produced maximum zone of inhibition of 8.5±0.4mm against *P. aerugenosa,* 7.3±0.45mm against *S. dysentery* and 7±0.2mm against *E. coli*. *K.pneumoniae* and *S. typhi* showed an average zone of inhibition of 6±0.11mm and 6.1±0.36mm by acetone crude leave extracts. Diethyl ether crude leave extract also inhibited *E. coli* with a maximum zone size of 11.8±0.28 mm and a least activity of 4±0.15mm against *P. aerugenosa.* Whereas hexane crude leaves extract did not exhibit any zone of inhibition against the tested human bacterial pathogens (Table.3)

Table 3 The effect of the different extracts of the leaves of *Justicia Schimperiana* against the bacterial test organism using disc diffusion method (Zones of inhibition; Mean± SD mm)

Test organisms	Mean zone of inhibition of leaves extract* *Justicia Schimperiana* (Mean± SD mm)				
	Methanol	Ethanol	Diethyl Ether	Acetone	Hexane
Escherichia coli	10.8±0.2	12.4±0.2	11.8±0.28	7±0.2	-
Salmonella typhi	8.7±0.26	14.7±0.3	-	6.1±0.36	-
Shigella dysentery	14.5±0.5	16±0.2	-	7.3±0.45	-
Staphylococcus aureus	6.7±0.2	13.1±0.28	-	6±0.2	-
Pseudomonas aeruginosa	6.4±0.17	10.8±0.26	4±0.15	8.5±0.4	-
Klebsiella pneumonia	7.7±0.25	14.8±0.2	6±0.11	6.4±0.4	-

NB: - implies no inhibition zone detected*; *= *a crude extract of at concentration of 100mg/ml was used for assay.

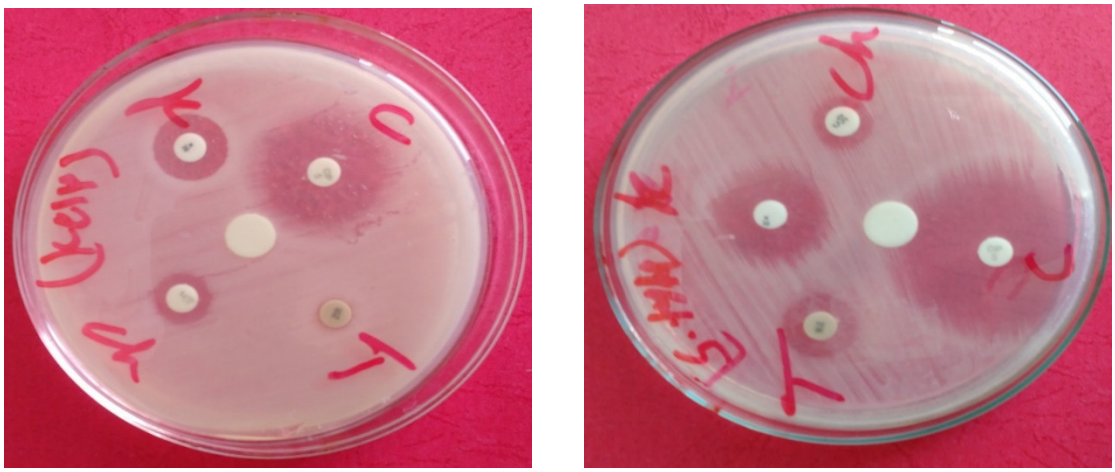
Inhibitory Zones of test pathogens with Standard Antibiotics (Positive control)

Four different antibiotics such as Ciprofloxin, Tetracycline, Kanamycin and Chloramphenicol were used as

standard and as positive control for the testing of antibacterial activity of six different human pathogens. The Ciprofloxin showed maximum zone of inhibition ranging from 20-35 against all pathogens; Kanamycin showed average zone of inhibition 20mm, Tetracycline exhibited ranging from 8-18mm and Chloramphenicol showed least inhibition against all test pathogens (Table. 4).

Table 4 The inhibition zone of antibiotics against human pathogens

Test organisms	Zone of inhibition in mm			
	Ciprofloxin	Kanamycin	Tetracycline	Chloramphenicol
Escherichia coli	30	20	15	10
Salmonella typhi	35	20	15	10
Shigella dysentery	32	20	13	10
Staphylococcus aureus	31	20	10	5
Pseudomonas aeruginosa	30	15	8	5
Klebsiella pneumonia	20	15	20	11



Activity of commercial antibiotics against human pathogens (photo taken after activates).

Minimum Inhibitory Concentration of Plant Extracts (MIC)

The MIC assay was employed to evaluate the efficacy of the plant crude leave extracts to inhibit the growth of bacterial test organisms. The crude leave extracts of two medicinal plants were subjected to the concentrations ranging from 1.56 mg/ml to 50mg/ml. In the antibacterial activity test, five different solvents were used for their *in vitro* antibacterial test among which only the best three solvents namely methanol, ethanol and acetone were selected for MIC test.

MIC of *R. chalepensis* crude leaves extracts against human pathogenic bacterial organisms

The methanol extracts of *R. chalepensis* showed inhibitory activity with MIC of 3.12 mg/ml against *S. aureus* and *P. aerugenosa,* followed by *E. coli* with MIC of 12.5 mg/ml and *S. typhi* at 25mg/ml. The MIC of ethanol extract of *R. chalepensis* was 1.56 mg/ml for *S. typhi* and 3.12 mg/ml for *E. coli* and *P. aerugenosa* respectively. Other pathogenic organisms *S. dysentry*, *S. aureus* and *K. pneumoniae* exhibited higher MICs. On the other hand, the acetone extract had MIC of 25 mg/ml against all the test pathogenic bacteria (Table 5).

MIC of *J. schimperiana* crude leaves extracts against human pathogenic bacterial organism

The methanol crude leave extracts of *J. Schimperiana* exhibited a strong MIC activity at 3.12 mg /ml concentration against *E. coli* and *S. dysentry* followed by *S. typhi* at 12.5 mg/ml and *K. pneumoniae* at 25 mg/ml concentration. The MIC of ethanol extract was 3.12 mg /ml against *S. dysentry* and at 6.25 mg /ml against *S. typhi* followed by *P. aerugenosa* and *K. pneumoniae* at 12.5 mg/ml concentration. The Acetone extracts of *J. Schimperiana* showed good inhibition result with MIC of 25 and 50 mg/ml against the pathogens *S. dysentry*, *S. aureus* and *K. pneumoniae* (Table 6).

Table 5 MIC of *Ruta chalapensis* crude leaves extracts against human pathogenic bacterial organism in mg/ml

R. chalapensis	Con mg/ml	*E. coli*	*S. typhi*	*S. dysentery*	*S. aureus*	*P. aeruginosa*	*K. pneumonia*
Methanol	1.56	-	-	-	-	-	-
	3.12	-	-	-	**	**	-
	6.25	-	-	-	+	+	-
	12.5	**	-	-	+	+	-
	25	+	**	-	+	+	**
	50	+	+	**	+	+	+
Ethanol	1.56	-	**	-	-	-	-
	3.12	**	+	-	-	**	-
	6.25	+	+	**		+	-
	12.5	+	+	+	**	+	-
	25	+	+	+	+	+	**
	50	+	+	+	+	+	+
Acetone	1.56	-	-	-	-	-	-
	3.12	-	-	-		-	-
	6.25	-	-	-	-	-	-
	12.5	-	-	-	-	-	-
	25	**	**	**	**	-	**
	50	+	+	+	+	-	+

**** = Minimum Inhibitory concentration + = Positive inhibition observed - = No activities (bacterial growth observed)**

Table 6 MIC of *Justicia Schimperiana* crude leaves extracts against human pathogenic bacterial organism in mg/ml

J. schimperiana	Con. mg/ml	*E. coli*	*S. typhi*	*S. dysentry*	*S. aureus*	*P. aeruginosa*	*K.pneumonia*
Methanol	1.56	-	-		-	-	-
	3.12	**	-	**	-	-	-
	6.25	+	-	+	-	-	-
	12.5	+	**	+	-	-	-
	25	+	+	+	-	-	**
	50	+	+	+	-	-	+
Ethanol	1.56	-	-	-	-	-	-
	3.12	-	-	**	-	-	-
	6.25	-	**	+	-	-	-
	12.5	-	+	+	-	**	**
	25	**	+	+	**	+	+
	50	+	+	+	+	+	+
Acetone	1.56	-	-	-	-	-	-
	3.12	-	-	-	-	-	-
	6.25	-	-	-	-	-	-
	12.5	-	-	-	-	-	-
	25	-	-	**	-	-	-
	50	-	-	+	**	**	**

** = Minimum Inhibitory concentration + = Positive inhibition observed - = No activities (bacterial growth observed)

DISCUSSIONS

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. Many reports are available on the antiviral, antibacterial, antifungal, anthelminthic, and anti-inflammatory properties of plants (Palombo and Semple, 2001, Kumarasamy *et al*., 2002, Stepanovic, *et al*., 2003, Bylka *et al*., 2004). Traditional medicine comprises of therapeutic practices that have been in existence, for hundreds of years, before the development and spread of modern medicine and are in use today. In the present study, crude extracts have been eluted from the leaves of two different Plants viz. *Justicia schimprena* and *Ruta chalepensis* using five different solvents such as methanol, diethyl ether, ethanol, acetone and hexane. The yield of the extract that was obtained by different solvents considerably differs in two of the medicinal plants (Table 1).

In these studies, among the solvents used to extract the biologically active substances from two medicinal plants, ethanol and methanol were the best solvents; followed by acetone and least by diethyl ether and hexane (Table 1). So, this displayed that the extraction of medicinal plants with different solvents may show different result which based on the potential of the solvents used to extract the biologically active constituents (George *et al*., 2010). In the present study, ethanol and methanol crude leaves extracts of *J. shimperna* and *R. chalepensis* showed the strongest activity against *E. coli, S. aureus, S. typhi,* and *S. dysentry* compared with other three solvents and plant based products have been effectively proven for their utilization as source for antimicrobial compounds. The ethanol extract of *Ruta chalepensis* showed conspicuous activity against the human bacterial pathogens. The highest zone of inhibition was observed in *Salmonella typhi* measuring around 15±0.5mm, followed by .*S. aureus* (13±0.11mm), *P. aerugenosa* (13±0.2mm) and *Escherichia coli* (12.5±0.5mm), which strengthens by Tadege *et al*., 2005 report. Moreover *R. chalpensis* has good inhibition for gram positive bacteria in all the extracts (Ahmed *et al*., 2010).

The present study showed that *S.typhi* was the most susceptible test organism for ethanol solvent extract leaves of *R. chalepensis*. The minimum inhibition (7.9±0.11mm) was observed against *K. pneumonia* by ethanol extraction. Similar extract was reported to have activity against the pathogenic *S. mutants* (Tadege *et al*., 2005). Diethyl ether extract exhibited moderate antibacterial activity of 7.1±0.32mm against *S. dysentery* and least activity against *K. pneumoniae (*5.2±0.2mm), and *P. aerugenosa* (5.1±0.28mm). In the present study, the MIC of ethanol extract of *R. chalepensis* was 1.56mg/ml against *S. typhi* and 3.12 mg/ml against *Escherichia coli* and *P. aerugenosa.* The result of the study clearly indicates that methanol and ethanol extracts are good in inhibiting the bacteria tested. Methanol extract of *J. schimperiana* on *S. dysentry* and *E. coli* was highly significant with a zone size of 14.5±0.5mm and10.8±0.2mm which is in agreement with Pavithra et al (2011) who reported that the methanol extracts of *Mollugo cerviana* inhibited the growth of *S. aureus and E. coli* with zones of 7.33±0.57 to 11±1, while chloroform extracts were ineffective against these bacterial strains.

The ethanolic extract of *J. schiperiana* was found to be the best which produced the maximum antibacterial activity against all the tested pathogens with the highest activity produced against *S. dysentery* with the zone size of 16±0.2mm, followed by *K. pneumoniae*14.8±0.2mm, *S. typhi* 14.7±0.3mm, *S. aureus*13.1±0.38 whereas Acetone extract produced a maximum zone of inhibition around 8.5±0.4mm against *P. aerugenosa*, 7.3±0.45mm against *S. dysentery* and 7±0.2mm against *E. coli*. Similar results have been reported by previous findings (Mahesh and Satish, 2008; Rajendran and Ramakrishnan, 2009; Sundaram *et al*., 2011. MIC activity at 3.12 mg /ml concentration of methanol extract of *J. schimprene* observed against *E. coli* and *S. dysentry* followed by *S. typhi*. The differences between the antibacterial activity of the two medicinal plant species show variations not only among different chemical extraction, but also among different species of plants; and in inherent resistance of the tested bacteria species (Nayan *et al*., 2011).

CONCLUSION

From the above results it could be concluded that the crude leave extracts of the two plants especially the ethanol and methanol revealed that they have higher potential to produce broad spectral antibacterial activity with minimal concentration against a wide range of human pathogens. The extracts were good in inhibiting *Escherichia coli, Salmonella typhi, Shigella dysentery, Pseudomonas aerugenosa* and in some instances *Klebssiella pneumoniae.* The results of this study provide an insight into the anti-microbial properties of the extracts of *Ruta chalepensis and Justicia schimperiana.* As well, it created an opportunity for phytoscreening of bioactive extracts for initial fractionation and further studies of these two medicinal plants in the antibacterial assays. This in vitro study demonstrated that these two folklore medicinal plants have good potential. This study gives an indication of the efficacy of the plants obtained from the traditional healersb and form a basis for further studies of the potent plants so as to isolate the bioactive compounds responsible for the antimicrobial activity.

Competing Interests

The authors declare that they have no competing interests.

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